EXHIBIT 2

lipid molecules move from one side of a membrane to the other? (2) How do phospholipid molecules move from one site to another within the cell? (3) How does phospholipid transport directed to specific organelles account for the differences in phospholipid composition of membranes within a single cell?

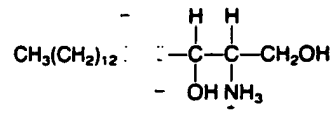
Investigations of transmembrane movement of phospholipids (question 1) use specific lipid probes that allow detection of a lipid on only one side of a bilayer. As mentioned in Tools of Biochemistry 10A, one such approach inv lves the use of a spin label, a lipid analog that is detectable from its electron paramagnetic resonance spectrum. Such measurements show that transbilayer movement, or "flip-flop," does occur spontaneously but is quite slow. Measurements in vivo show much faster transbilayer movement, so proteins or other factors may promote flip-flop in living cells.

Transport of phospholipids within the cell (question 2) involves largely the transfer of fragments of membranes of the ER into the Golgi complex, as was shown in Figure 19.1. Membrane vesicles are constantly pinched off from the Golgi, and these vesicles, containing secretory products, fuse with the plasma membrane for secretion of their contents via **exocytosis** (transport out of the cell). It seems likely that this route is used not only for extracellular secretion but also for transport of membrane lipids to the plasma membrane. Probably comparable processes transport membrane lipids to mitochondria, plant chloroplasts, and nuclei, although these processes are not as well understood.

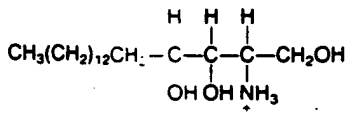
Membranes are assembled by membrane vesicles' moving from synthesis sites in the endoplasmic reticulum and Golgi complex to existing membranes and fusing with them.

To explain the variability of membrane lipid composition within a given cell (question 3), we can postulate the existence in Golgi membranes of specific targeting proteins—proteins that preferentially associate with certain lipids and have an affinity for certain organelles. Another mechanism involves the action of phospholipid exchange proteins—cytosolic proteins that bind a phospholipid and can catalyze its exchange with a corresponding membrane lipid. The protein-bound lipid moves into the membrane, and the membrane lipid becomes bound to the cytosolic protein. This mechanism does not provide for net transfer of lipid to a membrane, but it does allow for modulation of the lipid comp sition of a particular membrane.

METABOLISM OF SPHINGOLIPIDS



Sphingosine



Phytosphingosine

Interest in sphingolipids focuses largely on their important role in nervous tissue and, related to this role, a number of human genetic defects of sphingolipid metabolism. Sphingolipids are also widely distributed in the membranes of plant cells and in lower eukaryotes such as yeast.

Recall from Chapter 10 that sphingolipids are derivatives of the base *sphingosine*. Plant sphingolipids contain a slightly different form of this compound, called phytosphingosine. The sphingolipids include *ceramide* (*N*-acylsphingosine), *sphingomyelin* (*N*-acylsphingosine phosphorylcholine), and a family of carbohydrate-containing sphingolipids called neutral and acidic glycosphingolipids; the latter substances include *cerebrosides* and *gangliosides*. Ceramide serves as the precursor to both sphingomyelin and the glycosphingolipids.

In animals the pathway to ceramide starts with the synthesis of a sphingosine derivative, sphinganine, from palmitoyl-CoA and serine (Figure 19.12). After reduction of the resulting keto group, the amino group of sphinganine is acylated to give a ceramide. The sphinganine unit of this compound is then desaturated to give a ceramide with a sphingosine base. Transfer f a phosphocholine unit from phosphatidylcholine yields sphingomyelin plus diacylglycerol.

The pathways leading to glycosphingolipids are more numerous, but the metabolic strategies are comparable to those we have encountered bef re in synthesis

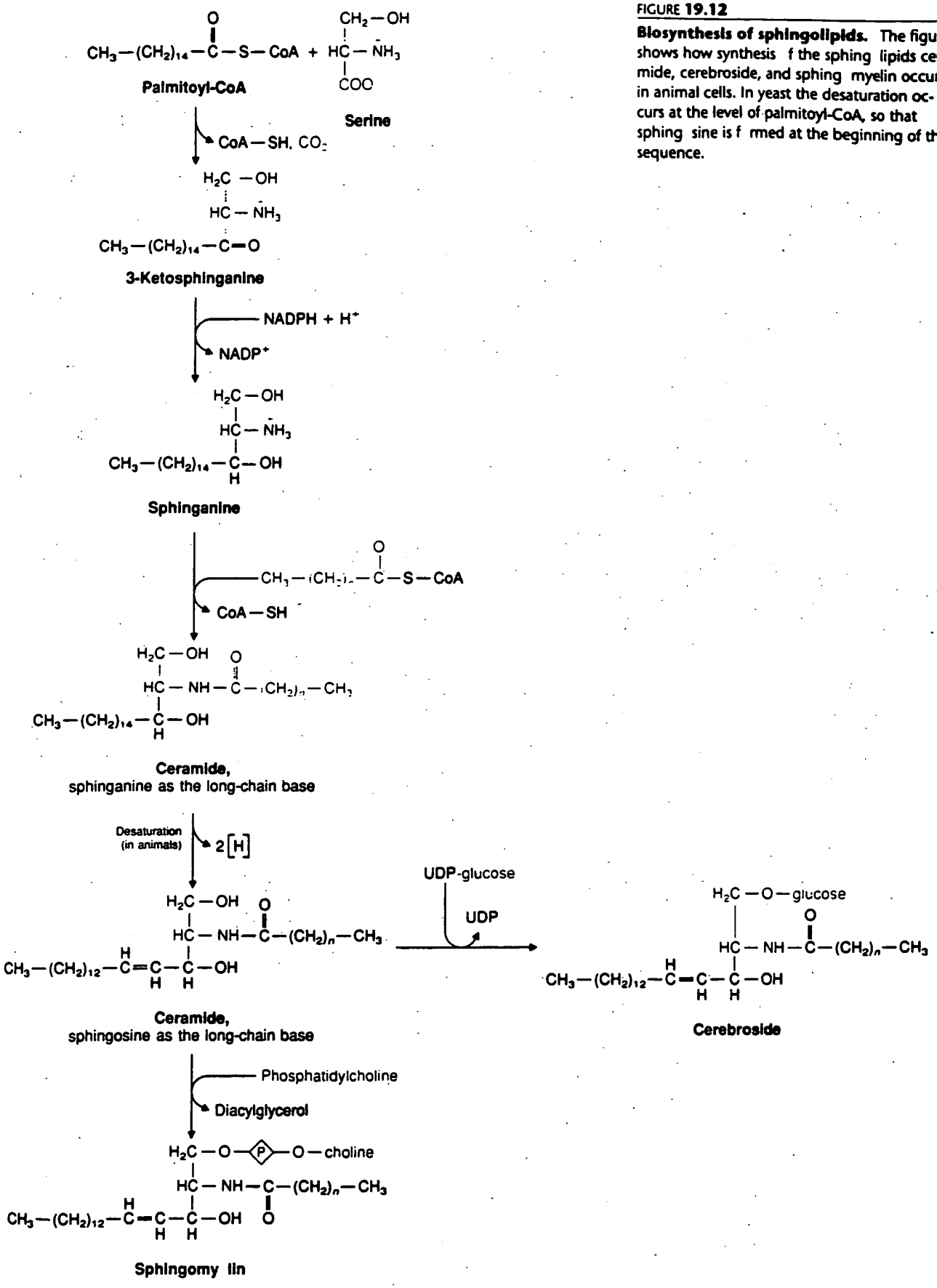


FIGURE **19.12**

Biosynthesis of sphingolipids. The figure shows how synthesis f the sphing lipids ceramide, cerebroside, and sphing myelin occurs in animal cells. In yeast the desaturation occurs at the level of palmitoyl-CoA, so that sphing sine is f rmed at the beginning of the sequence.

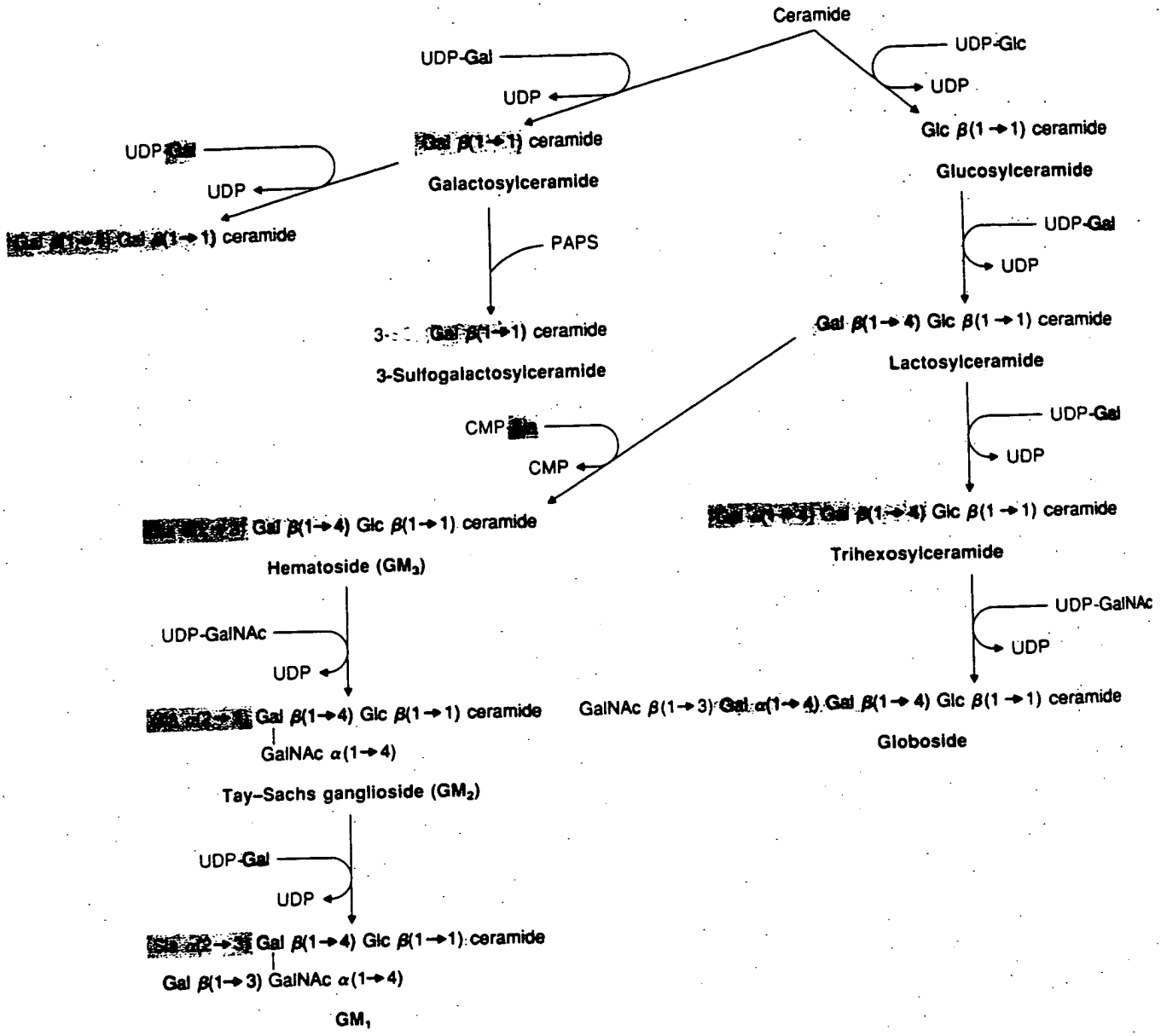


FIGURE **19.13**

Pathways of synthesis of glycosphingolipids. The common name of each compound is given. PAPS is a sulfate group donor (see Chapter 21).

Nucleotide-linked sugars and glycosyltransferases are involved in glycosphingolipid biosynthesis.

of the oligosaccharide chains of glycoproteins (see Chapter 16). The pathways involve the stepwise addition of monosaccharide units, using nucleotide-linked sugars as the activated biosynthetic substrates and with ceramide as the initial monosaccharide acceptor. The sugar nucleotides involved in glycosphingolipid synthesis include UDP-glucose (UDP-Glc), UDP-galactose (UDP-Gal), UDP-*N*-acetylgalactosamine (UDP-GalNAc), and CMP-*N*-acetylneuraminic acid (CMP-Sia, or CMP-sialic acid). Figure 19.13 shows pathways leading to some of the most abundant glycosphingolipids.

Sphingolipids, especially sphingomyelin, are abundant components of the *myelin sheath*, a multilayered structure that protects and insulates cells of the central nervous system (Figure 19.14). In human myelin, sphingolipids constitute some 25% of the total lipid. Sphingolipids are in a continuous state of metabolic turnover, both synthesis and degradation. Degradation occurs in the lysosomes, by a family of hydrolytic enzymes. These pathways are of great medical interest because of their relationship to a group of congenital diseases called sphingolipi-

TABLE 19.1 Inherited diseases of sphingolipid catabolism

Disease	Defective Enzyme[a]	Accumulated Intermediate
GM_1 gangliosidosis	β-Galactosidase	GM_1 ganglioside
Tay–Sachs disease	β-*N*-Acetylhexosaminidase A	GM_2 (Tay–Sachs) ganglioside
Fabry's disease	❸ α-Galactosidase A	Trihexosylceramide
Gaucher's disease	❹ β-Glucosidase	Glucosylceramide
Niemann–Pick disease	❺ Sphingomyelinase	Sphingomyelin
Farber's lipogranulomatosis	❻ Ceramidase	Ceramide
Globoid cell leukodystrophy	❼ β-Galactosidase	Galactosylceramide
Metachromatic leukodystrophy	❽ Arylsulfatase A	3-Sulfogalactosyl-ceramide
Sandhoff disease	❾ *N*-Acetylhexosaminidases A and B	GM_1 ganglioside and globoside

[a]Numbers refer to enzymes shown in Figure 19.15.

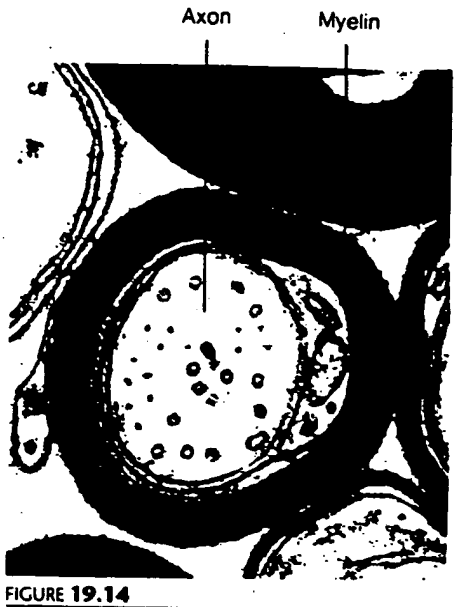


FIGURE **19.14**

A myelinated axon from the spinal cord. Myelin, an insulating layer wrapping about the axon, is rich in sphingomyelin.

Courtesy of Dr. Cedric Raine.

doses (also known as lipid storage diseases). Each condition is characterized by deficiency of one of the degradative enzymes, with concomitant accumulation within the lysosome of the substrate for the deficient enzyme (Table 19.1). In fact, structural analysis of the abnormal metabolites that accumulate helped to establish the degradative pathways, which are depicted in Figure 19.15. Most of these diseases are autosomal recessive, which means that two defective alleles of the gene encoding a particular enzyme must be present in an individual for disease symptoms to be manifest. Because of the large amounts of sphingolipids in nervous tissue, it is perhaps not surprising that most of the sphingolipidoses involve severely impaired central nervous system function.

The best known of the sphingolipidoses is Tay–Sachs disease, originally described in 1881, which is a deficiency of the lysosomal *N*-acetylhexosaminidase A. The enzyme deficiency causes accumulation of the ganglioside called GM_2, particularly in the brain. The disease is devastating, causing nervous system degeneration, mental retardation, blindness, and death, usually by the age of four.

Although Tay–Sachs disease is rare in the general population, the defective gene is relatively common among Ashkenazic Jews (those of middle and eastern European extraction). Among American Jews, about 1 in 30 individuals carries the defective gene. Thus, two Jewish parents carry an appreciable risk of bearing a Tay–Sachs child. Because there is no known cure for the disease, attention has focused on prenatal detection. In fact, this was one of the first genetic diseases to be successfully diagnosed in conjunction with amniocentesis. At present the only known way to deal with Tay–Sachs disease, when it is detected in this way, is to terminate the pregnancy. In countries that carry out screening for the homozygous state, followed by abortion when necessary, the incidence of the disease has dropped virtually to zero.

Little is known about specific biochemical functions of sphingolipids, but their presence in the outer surface of plasma membranes of animal cells provides some tantalizing clues. Gangliosides are receptors for specific agents, such as cholera toxin, which binds to ganglioside GM_1, or influenza virus, which recognizes the sialic acid portion of certain gangliosides. The true roles of these gangliosides are unknown. Also, substantial changes in the glycolipid content of cell surfaces become evident after those cells undergo oncogenic transformation. Here, research attention focuses on both the mechanism of such changes and the

Genetic defects in glycosphingolipid catabolism cause breakdown intermediates to accumulate in nervous tissue, with severe consequences.

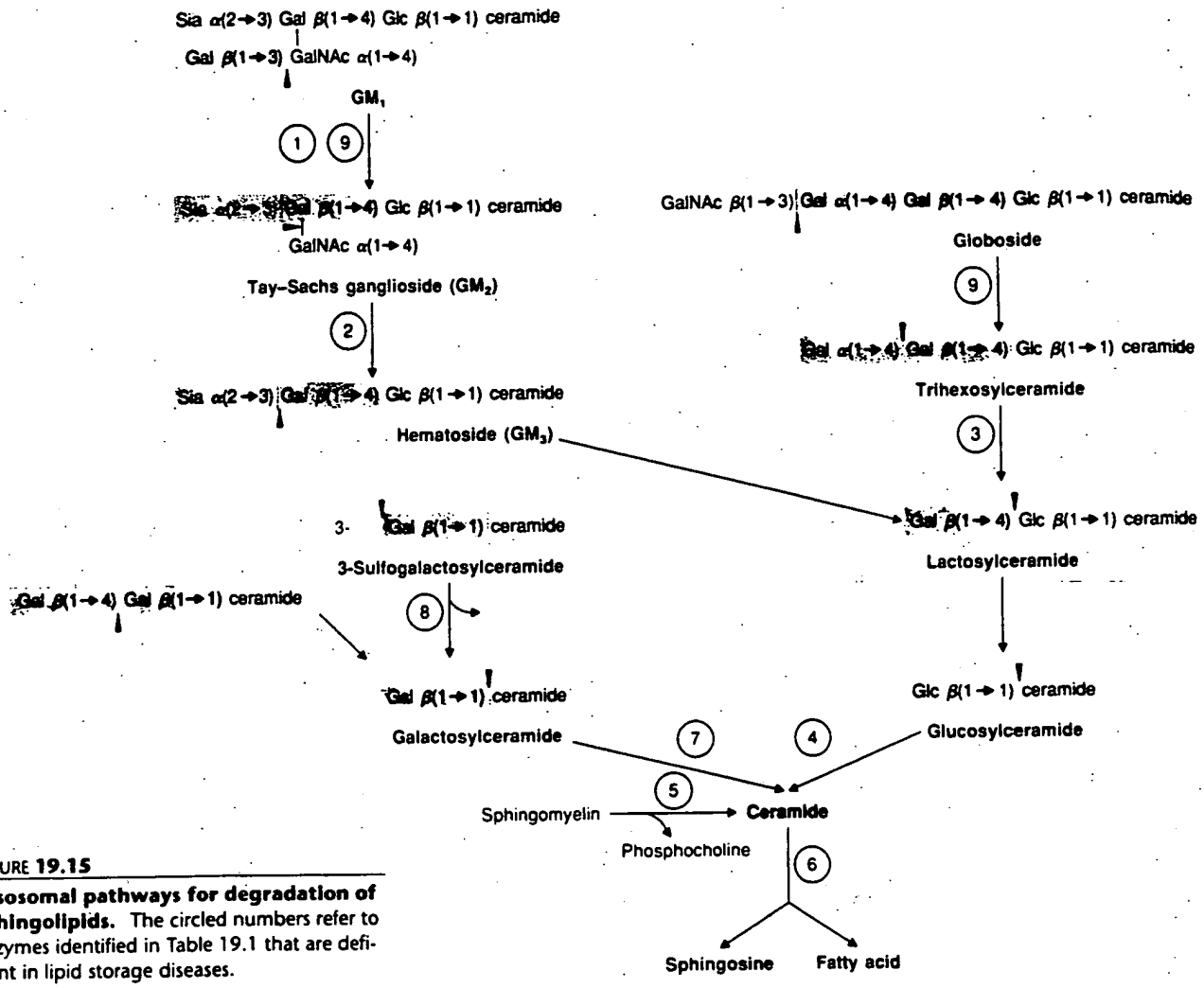


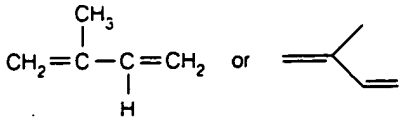
FIGURE **19.15**

Lysosomal pathways for degradation of sphingolipids. The circled numbers refer to enzymes identified in Table 19.1 that are deficient in lipid storage diseases.

potential for understanding the extent to which these changes are responsible for the altered properties of tumor cells, such as tumorigenesis, or the loss of growth control, adhesivity, or antigenic specificity.

STEROID METABOLISM

We turn now to an extraordinarily large and diverse group of lipids, the isoprenoids, or terpenes. These compounds are built up from one or more five-carbon units, activated derivatives of isoprene. The family includes steroids and bile acids; the lipid-soluble vitamins; the dolichol and undecaprenol phosphates we encountered in glycoprotein synthesis; phytol, the long-chain alcohol in chlorophyll; gibberellins, a family of plant growth hormones; insect juvenile hormones; the major components of rubber; coenzyme Q; and many more compounds.



Isoprene

Much of our discussion of isoprenoids will focus on a single steroid compound, cholesterol. As discussed in Chapter 10, this lipid is a major component of animal cell membranes, where it participates in modulation of membrane fluidity. In animals it also serves as precursor to all of the steroid hormones, to vitamin D, and to the bile acids, which aid in fat digestion. And as we discussed in Chapter 18, there is intense medical interest in cholesterol because of the relationships among diet, blood cholesterol levels, atherosclerosis, and heart disease.

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